this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor (*T*), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$=Width of peak at 5 percent height; and
f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (*n*) of the column as follows:

$$n = 5.545 \left[\frac{t_R}{W_h} \right]^2$$

where:

n=Efficiency, as number of theoretical plates for column;
t_R=Retention time of solute; and
w_h=Peak width at half-height.

(3) *Resolution.* Calculate the resolution (*R*) as follows:

$$R = \frac{2(t_{RJ} - t_{Ri})}{w_i + w_J}$$

where:

t_{RJ}=Retention time of a solute eluting after *i* (t_{RJ} is larger than t_{Ri});
t_{Ri}=Retention time of any solute;
w_i=Width of peak at baseline of any solute; and
w_J=Width of peak at baseline of any solute eluting after *i.*

(4) *Coefficient of variation (Relative standard deviation).*

Calculate the coefficient of variation (S_R) in percent) as follows:

$$\underline{S_R} = \frac{100}{\overline{\underline{X}}} \left[\frac{\sum_{i=1}^{N} (\underline{X_i} - \overline{\underline{X}})^2}{\underline{N} - 1} \right]^{\frac{1}{2}}$$

where:

X is the mean of *N* individual measurements of X_i. If the complete operating system meets the system suitability require-

ments of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, using the sample solution in lieu of the working standard solution.

§ 436.364 Atomic absorption test for sodium carbonate content of cefmenoxime hydrochloride for injection.

(a) *Apparatus.* A suitable atomic absorbance spectrophotometer equipped with:

(1) A suitable sodium hollow-cathode discharge lamp;

(2) An oxidizing air-acetylene flame;

(3) A nebulizer-burner system;

(4) An optical dispersing device capable of isolating a resonance line of sodium from other wavelengths produced by the emission source; and

(5) A suitable radiation detector.

(b) *Reagents.* Ionization buffer: Dissolve 19.07 grams of potassium chloride in distilled water and dilute to 1,000 milliliters.

(c) *Preparation of reference standard and sample solutions*—(1) *Reference standard solution.* Accurately weigh approximately 140 milligrams of sodium chloride which has been previously dired for 40 to 50 minutes at a temperature of 500 to 650 ° C. Dissolve and dilute with sufficient distilled water to obtain a stock solution containing 5.5 micrograms of sodium per milliliter. Mix 10 milliliters of the stock solution with 10 milliliters of ionization buffer and dilute the mixture with distilled water to obtain a solution containing 0.55 microgram of sodium per milliliter.

(2) *Sample solution.* Dilute the sample solution used in § 442.222(b)(1)(ii)(B)(*I*) of this chapter, with sufficient distilled water to obtain a stock solution containing 5.5 micrograms of sodium per milliliter (estimated). Mix 10 milliliters of the stock solution with 10 milliliters of ionization buffer and dilute the mixture with distilled water to obtain a solution containing 0.55 microgram of sodium per milliliter (estimated).

(3) *Procedure.* Determine the atomic absorbance of the reference standard and sample solutions at a wavelength

of 589 nanometers, using the atomic absorbance spectrophotometer and a reagent blank prepared by diluting 10 milliliters of ionization buffer to 100 milliliters with distilled water.

(d) *Calculations.* Calculate the percent sodium carbonate as follows:

$$\text{Percent sodium carbonate} = \frac{A_\text{u} \times P_\text{s} \times 100 \times 0.9068 \times d}{A_\text{s} \times C_\text{u}}$$

where:

A_u=Absorbance of sodium in the sample solution;

A_s=Absorbance of sodium in the reference standard solution;

P_s=Milligrams of sodium chloride per milliliter of the reference standard solution;

C_u=Milligrams of sample per milliliter of sample solution; and

d= Dilution factor of the sample.

[53 FR 13401, Apr. 25, 1988]

§ 436.365 Thin layer chromatographic identity test for rifampin.

(a) *Equipment*—(1) *Chromatography tank.* Use a rectangular tank approximately 23×23×9 centimeters, with a glass solvent trough on the bottom and a tight-fitting cover, lined with Whatman #3MM chromatographic paper or equivalent.

(2) *Plates.* Use 20×20 centimeter thin layer chromatography plates coated with silica gel 60 F–254 or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix chloroform and methanol in volumetric proportions of 90:10, respectively.

(c) *Spotting solutions*—(1) *Preparation of working standard solution.* Dissolve approximately 50 milligrams of rifampin working standard in 5 milliliters of chloroform.

(2) *Preparation of sample solution.* Dissolve the contents of a sample vial in 60 milliliters of chloroform.

(d) *Procedure.* Pour the developing solvent into the glass trough on the bottom of the tank and onto the paper lining the walls of the tank. Cover and seal the tank. Allow it to equilibrate. Prepare a plate as follows: On a line 2.5 centimeters from the base of the thin layer chromatography plate and at intervals of 2.0 centimeters, spot 3 microliters of the working standard solution to points 1 and 3. When these spots are dry, apply 3 microliters of the sample solution to points 2 and 3. After all the spots are thoroughly dry, place the plate into the trough in the bottom of the tank. Cover and tightly seal the tank. Allow the solvent front to travel about 7 centimeters from the starting line. Remove the plate from the tank and air dry.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line, and the distance the red spots are from the starting line. Divide the latter by the former to calculate the R_f value.

[54 FR 38375, Sept. 18, 1989; 54 FR 42886, Oct. 18, 1989]

§ 436.366 High-performance liquid chromatography assay for determining chromatographic purity of vancomycin.

(a) *Apparatus.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers or preferably 280 nanometers;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) A 25-centimeter analytical column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles; 5 micrometers in diameter.

(b) *Reagents*—(1) *0.2 percent triethylammonium phosphate buffer.* To 2,000 milliliters of distilled water, either add 4 milliliters of triethylamine or 4 grams of triethylammonium chloride. Adjust the pH to 3.2 with phosphoric acid.

(2) *Sample solvents.* (i) Vancomycin hydrochloride: Mobile Phase A.